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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/02, C12N 9/50, 15/57 A61K 39/02, 39/095	A1	(11) International Publication Number: WO 90/11367 (43) International Publication Date: 4 October 1990 (04.10.90)
(21) International Application Number: PCT/DK90/00073 (22) International Filing Date: 16 March 1990 (16.03.90) (30) Priority data: 1308/89 17 March 1989 (17.03.89) DK (60) Parent Application or Grant (63) Related by Continuation US 324,750 (CIP) Filed on 17 March 1989 (17.03.89) (71)(72) Applicants and Inventors: KILIAN, Mogens [DK/DK]; Lindevangsvej 15, DK-8240 Risskov (DK). POULSEN, Knud [DK/DK]; Kirketoften 5, DK-9260 Viby J. (DK).		(74) Agent: HOFMAN-BANG & BOUTARD A/S; Adelgade 15, DK-1304 Copenhagen K (DK). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), + CH, CH (European patent), CM (OAPI patent), DE, + DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (Eu- ropean patent), JP, KP, KR, LK, LU, LU (European pa- tent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i>
(54) Title: IMMUNOGLOBULIN A1 PROTEASES (IgA1 PROTEASES), METHOD OF GENE-TECHNOLOGICALLY PRODUCING THIS TYPE OF ENZYME AND VACCINES CONTAINING THE ENZYMES AND FRAG- MENTS THEREOF FOR IMMUNIZING AGAINST BACTERIAL MENINGITIS AND OTHER DISEASES CAUSED BY IgA1 PROTEASE-PRODUCING BACTERIA		
(57) Abstract <p>Secretion of IgA1 proteases is a characteristic of Haemophilus influenzae and several other bacterial pathogens <u>causing infectious diseases</u>, including meningitis. Indirect evidence suggests that the proteases are important virulence factors. The iga gene <u>encoding</u> IgA1 protease, especially that from H. influenzae serotype b, has been cloned into Escherichia coli in which the recombinant iga gene was expressed and the resulting protease secreted. Sequencing a part of a 7.5 kb DNA fragment containing the iga gene revealed a large open reading frame (ORF) with a strongly biased codon usage and having the potential of encoding a protein of 1541 amino acids and a molecular mass of 169 kd. Putative promoter and terminator elements flanking the ORF have been identified. Comparing the deduced amino acid sequence of the produced H. influenzae IgA1 protease with that of a similar protease from Neisseria gonorrhoeae reveals several domains with a high degree of homology. Analogous to mechanisms known from the N. gonorrhoeae IgA protease secretion, there is proposed a scheme of post-translational modifications of the H. influenzae IgA1 protease precursor leading to a secreted protease with a molecular mass of 108 kd which is close to the M_r 100 kd estimated for the mature IgA1 protease.</p>		

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Immunoglobulin A1 proteases (IgA1 proteases), method of gene-technologically producing this type of enzyme and vaccines containing the enzymes and fragments thereof for immunizing against bacterial meningitis and other diseases caused by IgA1 protease-producing bacteria

The present invention relates to immunoglobulin A1 proteases (IgA1 proteases) and fragments thereof and also to a method of gene-technologically producing the inventive IgA1 protease by cloning the gene for the IgA1 protease in question from a micro-organism, in particular Haemophilus influenzae serotype b, in an E. coli host organism from which the IgA1 protease formed is secreted extracellularly. The inventive IgA1 proteases can be used in vaccines, in particular for the prevention of meningitis, but also for immunizing against allergic diseases, gonorrhoea and other diseases caused by IgA protease-producing bacteria.

20

Background of the invention:

The immunoglobulin A1 proteases (IgA1 proteases) are extracellular bacterial enzymes which specifically cleave the heavy chain of human IgA1 molecules in the hinge region. Production of IgA1 protease seems to be correlated with the ability of the bacteria to infect and in some instances invade mucosal membranes. Notably, the three leading causes of bacterial meningitis (Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae) secrete IgA1 proteases whereas closely related non-pathogenic species are devoid of similar enzyme activity. IgA1 protease production is also a property of certain bacterial species causing vaginal and urinary tract infections, such as Neisseria gonorrhoeae and Ureaplasma urealyticum. In addition, several oral bacteria

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involved in dental plaque formation (*Streptococcus sanguis*, *Streptococcus oralis*, and *Streptococcus mitis* biovar 1 (the two latter species are former parts of *Streptococcus mitior*) and in the pathogenesis of

5 periodontal diseases (*Bacteroides* and *Capnocytophaga* species) secrete IgA1 proteases (Kilian, M. and Reinholdt, J.: "Interference with IgA defence mechanisms by extra-cellular enzymes", p. 173-208 in C.S.F. Easmon and J. Jeljaszewics (ed.), 1986, Medical Microbiology vol. 5,

10 Academic Press, Inc., London; see also Mulks, M. H.: "Microbial IgA proteases" p. 81-104 in I. A. Holder (ed.), 1985, Bacterial Enzymes and Virulence, CRC Press, and Plaut, A.G., Ann. Rev. Microbiol. 37, 603-622 (1983)). IgA1 proteases are believed to be important virulence

15 factors as they allow bacteria to interfere with the protective mechanisms of IgA1, which is the predominant immunoglobulin class on relevant mucosal surfaces (Kett, K. et al., J. Immunol. 136, 3631-3635 (1986)).

20 Because of the specificity of IgA1 proteases for human IgA1 experiments with animals cannot confirm the pathogenic significance of these enzymes. However, extensive cleavage of IgA in secretions and other body fluids from patients infected with said bacteria is observed. Further-

25 more, it has been shown that nasopharyngeal secretions from children with a history of allergic diseases, more than from healthy children, show extensive IgA1 protease-induced degradation of the IgA, which was to form a protective barrier to potential allergens and micro-

30 organisms (Christensen, C.H. and Kilian, M., Acta Path. Microbiol. Immunol. Scand. 92C, 85-87, 1984).

Several enzymatically different types of IgA1 proteases have been reported, each cleaving the human IgA1 molecule

35 at a specific site in the hinge region, as shown in fig. 1 (Kilian, M. and Reinholdt, J., supra). In *Haemophilus*

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influenzae at least two such distinct enzymes are known (type 1 cleaving the PRO-SER paptide bond at position 231-232 and type 2 cleaving the PRO-SER peptide bond at position 235-236). Likewise, *Neisseria meningitidis* and
5 *Neisseria gonorrhoeae* may secrete a type 1 or a type 2 protease (see fig. 1).

Polymorphism of IgA1 proteases:

10 IgA1 proteases from different bacteria have been compared by genetical and serological methods. Hybridization experiments using the bulk of a gonococcal IgA1 protease gene as probe revealed substantial homology (>78%) between
15 IgA1 protease genes from different gonococcal and meningococcal strains. More surprisingly, an estimated 67-75% homology between the gonococcal gene and that from a *Haemophilus influenzae* strain (Rd) was observed (Koohey, J.M. and Falkow, S., *Infect. Immun.* 43, 101-107, 1984). However, the use of restriction enzyme mapping of IgA1
20 protease genes revealed a considerable degree of polymorphism even between strains belonging to the same species (Halter, R. et al. *EMBO J.* 3, 1595-1601, 1984; Bricker, J. et al., *Infect. Immun.* 4, 370-374, 1985; Mulks, M.H. and Knapp, J.S., *Infect. Immun.* 55, 931-931,
25 1987). Within a large collection of *Haemophilus influenzae* serotype b strains four different IgA1 protease gene types were detected with restriction enzyme analyses (Poulsen, K., Hjorth, J.P. and Kilian, M. *Infect. Immun.* 56, 987-992, 1988).

30 The extensive polymorphism has been confirmed by comparing IgA1 proteases with neutralizing antibodies raised in rabbits. When immunized with IgA1 protease preparations rabbits respond with antibodies capable of neutralizing
35 the enzyme used for immunization. With such antibodies at least 15 antigenically different types of IgA1 proteases

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have been identified among *H.influenzae* isolates (Kilian, M. et al., *Molec. Immunol.* 20, 1051-1058, 1983) and two types among *H.influenzae* serotype b (Poulsen K. et al. supra). A partial antigenic relationship has been observed between one out of two strains of *N.meningitidis* and one strain of *N.gonorrhoeae* (Kilian, M., et al., *Ann. N.Y. Acad. Sci.* 409, 612-624, 1983). Furthermore, type 1 and type 2 IgA1 proteases of *Neisseriae* can be distinguished on the basis of their relative inactivation by sera from patients recovering from meningococcal infection by either type 1 or type 2 protease producing bacteria (Stafford and Plaue, *Abstr. Annu. Met. Am. Soc. Microbiol.* 1982, B125, p. 38).

Cloning of IgA1 protease genes

The genes encoding IgA1 protease (iga genes) produced by *Neisseria gonorrhoeae* type 1 (Fishman, Y., Bricker, J., Gilbert, J.V., Plaut, A.G. and Wright, A.: "Cloning of the type 1 immunoglobulin A1 protease from *Neisseria gonorrhoeae* and secretion of the enzyme from *Escherichia coli*", 1985, p. 164-168 in G. K. Schoolnik (ed.), *The pathogenic Neisseria*, Am. Soc. Microbiology, Washington DC) and type 2 (Koomey, J. M., Gill, R.E. and Falkow, S., *Proc. Natl. Acad. Sci. USA*, 79, 7881-7885, 1982; Halter, R., Pohlner, J. and Meyer, T.F., *EMBO, J.* 3, 1595-1601, 1984), *Haemophilus influenzae* type 1 of serotype d origin (Bricker, J. et. al., *Proc. Natl. Acad. Sci. USA*, 80, 2681-2685, 1983; and Koomey, J. M. and Falkow, S., supra) and type 2 of serotype C origin (Grundy, J. F. et. al., *J. Bacteriol.* 169, 4442-4450, 1987) *Neisseria meningitidis* (Koomey and Falkow, supra and *Streptococcus sanguis* (Gilbert, J.V. et al., *Infect. Immun.* 56, 1961-1966 (1988)) have been cloned.

E. coli transformed with each of these iga genes express

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IgA1 protease activity and the *E. coli* hosts, which harbour the *H. influenzae* and the *N. gonorrhoeae* *iga* genes, have been found to excrete the protease. At present, an example of the type 2 IgA1 protease gene from

5 *N. gonorrhoeae* is the only *iga* gene for which the nucleotide sequence is known (Pohlner, J. et al., *Nature* 325, 458-462 (1987); see also DE OS 36 22 221). The deduced primary structure of the protein together with analyses of intermediate IgA1 protease precursors have

10 revealed that the protease is expressed as a preprotein containing a signal peptide, the structural protease and a helper protein, which is autoproteolytically processed during the secretion process. Despite the similarity between the enzymes from *Neisseria* sp. and *H. influenzae* it

15 has been concluded that the *Haemophilus* enzyme is not transposed by the mechanism used for secretion of the *Neisseria* IgA1 protease (Fishman et. al., *supra*; see also DE OS 36 22 221).

20 It has now surprisingly been found that the gene for IgA1 protease from *Haemophilus influenzae* can be cloned in *E. coli* to achieve extracellular enzyme secretion, thus making it possible to extract the enzyme from the cultivation medium.

25 Previously, fragments of the cloned type 1 IgA1 protease gene of *H. influenzae* serotype d origin have been used as probes in Southern blot experiments to study the restriction site polymorphism of the *iga* gene among

30 different *H. influenzae* serotype b strains (Poulsen, K., Hjort, J.P. and Kilian, M., *Infect. Immun.* 56 987-992 (1988)). Based upon these *iga* gene restriction patterns the strains could be divided into four groups, which correlated with previously observed clusters of multilocus

35 genotypes (electrophoretic types). Three of the *iga* gene restriction types, which appeared to represent 98% of the

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H. influenzae serotype b population, exclusively contained strains of the same unique IgA1 protease "inhibition type" and therefore could form the basis for the development of a vaccine against H. influenzae meningitis.

5

Experiments with inhibitory antibodies have shown that the three gene types direct the production of proteases with a mutual epitope, which is found in each of the three proteases.

10

Detailed Description of Aspect 1

In the present invention H. influenzae strain HK368 was chosen as representative for the most common serotype b iga gene type (Poulsen, K. et al., supra). In the following the cloning and sequencing of the iga gene from this strain is described. Comparing the deduced amino acid sequence of this protease to that of N. gonorrhoeae reveals interesting regions of homology.

20

In the following the invention is illustrated in more detail, reference being made to the drawing in which

fig. 1 shows the primary structure of the hinge region of human IgA1;

25

fig. 2 shows the structure of the H. influenzae serotype b strain HK368 IgA1 protease gene;

fig. 3 shows the nucleotide sequence of the cloned iga gene from H. influenzae serotype b strain HK368;

30

fig. 4 illustrates the amino acid homologies between the deduced H. influenzae iga gene product and the N. gonorrhoeae iga gene product, and

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Fig. 5 shows a physical map of lambda HF13_{iga}-1 expressing the meningococcal IgA1 protease gene from strain HF13.

5 The invention is illustrated in more detail in the following. When carrying out the experiments the following materials and methods were used:

Bacterial strains. The wild-type H. influenzae serotype b
10 strain HK368 isolated from cerebrospinal fluid from a meningitis patient was the source of whole-cell DNA for molecular cloning. It was grown in Brain Heart Infusion broth (Difco, Detroit, Mich.) supplemented with hemin and nicotinamide adenine dinucleotide (NAD) (Kilian, M. J.
15 Gen. Microbiol. 93, 9-62 (1976)).

E. coli strain K802 was used for propagation of lambda phages. The M13mp19 phage and recombinant derivatives
20 hereof were propagated in E. coli JM109 as described by Yanisch-Perron, C. et al., Gene 33, 103-119 (1985).

Enzymes and chemicals. Restriction endonucleases were purchased from Amersham International (Amersham, England) and Boehringer GmbH (Mannheim, FRG). T4 ligase, Proteinase
25 K, RNase A, and DNase I were obtained from Boehringer; DNA polymerase I, DNA polymerase I Klenow fragment, and radioactively labelled deoxynucleotides were from Amersham. Exonuclease III came from Pharmacia (Uppsala, Sweden), lysozyme from Sigma (St. Louis, MS), M13
30 pentadecamer primer from New England Biolabs (Beverly, CA), and Sequenase DNA sequencing kit was from United States Biochemical Corporation (Cleveland, OH).

35 Cloning of H. influenzae HK 368 DNA in phage λ L47.1. DNA from H. influenzae isolated as described previously

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(Poulsen, K. et al., supra) was partially digested with Sau3A and fractionated by agarose gel electrophoresis. Fragments ranging in size from 12 to 20 kilobases (kb) were extracted from the gel by electroelution and cloned using λ L47.1 as BamHI substitution vector.

Phages packaged in vitro as described by Maniatis et al., "Molecular Cloning. A laboratory Manual", Cold Spring Harbor Laboratory, N.Y., were plated on E. coli strain K802 and positive plaques identified by in situ hybridization. Positive plaques were purified by replating on E. coli strain K802 and DNA isolated from 20 ml phage cultures (Mikkelsen, B.M. et al., Biochem. Genet. 23, 511-524 (1985)).

Nucleic acid hybridizations and DNA manipulations. As label in the hybridizations (α - 32 P)-dATP, which was incorporated into the DNA probes by nick-translation, was used (Rigby, P.W.J. et al., J. Mol. Biol. 11, 237-251 (1977)). The in situ probing of phages fixed on nitrocellulose filters was carried out as described by Benton and Davies, Science 196, 180-182 (1977) and Southern blot experiments were performed as described previously (Poulsen, K. et al., supra). Fragments of plasmid pVD116 containing H. influenzae type d iga gene were used as probes. Subcloning into M13mp19 of restriction fragments isolated by electroelution and DNA purifications and manipulations were carried out as described by Maniatis et al., supra.

Progressive deletions of recombinant M13mp19 phages for the sequencing procedure were produced by varying the time length of Exonuclease III digestion of BamHI/SacI opened replicative form DNA (Yanisch-Perron et al., supra). For removal of the resulting single stranded ends S1 nuclease was used instead of Exonuclease VII.

DNA sequencing. The sequences of individual clones were determined by the dideoxynucleotide chain termination method (Sanger, F. et al., Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977) and Biggin, M.D. et al., *ibid.* 80, 3963-3965 (1983)) using the 15-mer universal primer and (α - 35 S)-dATP. For some of the sequences Sequenase DNA sequencing kit was used according to the manufacturer's recommendations. The program of Larson and Messing (Nucl. Acids Res. 10, 39-49 (1982)) was used to assemble to sequence data using an Apple IIe computer.

Sequence alignment. Computer assisted analysis of amino acid sequence data was performed by the program described in J. Mol. Biol. 195, 43-61 (1987).

Assay for IgA1 protease activity. IgA1 protease activity in phage lysates was detected by mixing 1 vol. of the lysate supernatant with 1 vol. of a 2 mg/ml solution of hyman myeloma IgA1. After overnight incubation, cleavage of the substrate IgA1 was demonstrated by immunoelectrophoresis as described by Kilian, M. et al., Mol. Immunol. 220, 1051-1058 (1983)).

Immunization and inhibition assay. The supernatant of a E. coli lysate of a recombinant phage containing the iga gene was used for immunization of a rabbit. The immunization procedure and protease inhibition assay was as described previously (Kilian, M. et al., *supra*).

The following results were obtained:

Cloning of the H. influenzae serotype b iga gene in E. coli.

The E. coli plasmid pVD116 contains the iga gene from H.

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influenzae strain Rd⁻/b⁺ (Koomey, J. M., Proc. Natl. Acad. Sci. USA 79, 7881-7885 (1982)). A 2.0 kb ClaI/PstI fragment of this plasmid containing the 5' end of the iga gene of serotype d origin was used as radioactively
5 labelled probe to isolate the iga gene from a lambda-phage library of H. influenzae serotype b strain KH368 DAN using the vector λ L47.1. Replica filters of approximately 2×10^{-3} recombinant λ -phages from the non-amplified library were screened with the probe. Sixteen positive clones labelled
10 λ 368iga-1 to λ 368iga-16 were found. Assuming that iga is a single copy gene, this frequency is in acceptable agreement with the estimated H. influenzae genome size of 1.8×10^{-3} kb and an average insert of 15 kb in the recombinant λ -phages. Eleven of these positive clones were
15 purified and recombinant phage DNA isolated. The localization of recognition sites for the restriction endonucleases EcoRI and HindIII in the insert DNA were determined by complete and partial digestions of DNA from the individual λ 368iga phages as previously described
20 (Mikkelsen, B.M. et al., supra). In addition, DNA from λ 368iga-8 and λ 368iga-16 were mapped with respect to the restriction enzymes BamHI, ClaI, and PstI. The restriction maps of the inserts overlap are shown in Fig. 2. This strongly suggests that the iga gene in H. influenzae
25 strain KH368 is a single copy gene.

Localization of the iga gene. The previously described 5'-iga specific probe together with a 3'-iga specific 2.8 kb PstI/EcoRI fragment of pVD116 were hybridized to replica
30 Southern blots of EcoRI, HindIII, and Sau3A restricted DNA from each of the eleven λ 368iga clones analysed. Thereby, the iga gene was deduced to be localized within a 7.5 kb EcoRI/HindIII fragment and oriented as shown in Fig. 2.

35 The restriction enzyme Sau3A sites at the insert vector junctions are preserved during the cloning. For seven of

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the eleven recombinant clones analysed the size and number of DNA fragments generated by Sau3A and hybridizing to the two iga probes were identical. The same pattern of bands was observed in Southern blots of Sau3A digested whole-cell DNA from H. influenzae strain HK368 hybridized with the same two probes (data not shown). This indicated that not rearrangements of the hybridizing region in these lambda phages had occurred during the cloning procedure. In addition, this result confirms that the same iga-specific sequence had been cloned in these phages.

Liquid lysates from these seven recombinant phage clones were found to possess IgA1 protease activity. This confirms the cloning of the iga gene. In the lysates from three of the eleven clones, i.e. λ 368iga-2, λ 368iga-4, and λ 368iga-5, IgA1 cleaving activity could not be detected. DNA from these three clones lacked some of the Sau3A fragments found in strain KH368 genomic DNA, which hybridized to the 3'-iga probe, and therefore only contain the 5' part of the iga gene (see Fig. 2). The clone λ 368iga-8, which encodes IgA1 protease activity, lacked some of the HK368 genomic Sau3A fragments hybridizing to the 5'-iga probe. This indicates that the plasmid pVD116, which was used as probe, contains sequences originating from the H. influenzae serotype d genome beyond the iga gene.

The 7.5 kb EcoRI/HindIII fragment of λ 368iga-16, which contains the iga gene, was subcloned into M13mp19. In the supernatant of liquid cultures of E. coli JM109 transformed with this recombinant M13 phage we detected IgA1 protease activity at a level comparable to the IgA1 cleaving activity found in the supernatant of H. influenzae strain HK368 cultures. Because the iga gene was cloned in the opposite direction of the lac Z gene in M13mp19 this result confirms that the H. influenzae iga

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gene is expressed in *E. coli*. It also indicates that the secretion mechanisms of the IgA1 protease function in *E. coli* since the M13 phages do not lyse the bacterial cells.

5 Crude extract from a λ 368iga-16 lysate was used to immunize rabbits. The immunoglobulin fraction of the resulting antisera had complete inhibitory effect on the IgA1 protease activity from *H. influenzae* strain HK368 culture supernatant. This shows that *H. influenzae* strain
10 HK368 does not secrete any IgA1 protease unrelated to the one encoded by the iga gene cloned in λ 368iga-16.

Nucleotide sequence of the iga gene. The 7.5 kb EcoRI/HindIII fragment of λ 368iga-16, which contains the
15 iga gene, occasionally was unstable when subcloned into M13mp19 and propagated in *E. coli* JM109. Therefore, in the sequencing strategy the internal PstI site was used to divide this fragment (see Fig. 2). The sticky ends of the 5.9 kb HindIII/PstI and the 1.6 kb PstI/EcoRI fragments of
20 λ 368iga-16 (Fig. 21) were made blunt ended by the Klenow enzyme and subcloned in both orientations into the HincII site of M13mp19. Deletion derivatives of these subclones were generated by nuclease Exo III digestion for various times of SacI/BamHI-opened replicative form DNA followed
25 by nuclease S1 treatment and self ligation. The 5091 nucleotide (nt) sequence of the 7.5 kb EcoRI/HindIII fragment presented in Fig. 3 was obtained by sequencing a total of 79 such deletion clones, which gave overlapping sequences of both strands in this central part of the
30 fragment. The sequence across the internal PstI site, i.e. at nt 4856 to nt 4861 in Fig. 3 was verified by subcloning into M13mp19 and sequencing 300 nucleotides from the 3' end of the 1640 base pair HhaI fragment ranging from nt 3349 to nt 4988.

35

The sequence revealed a large open reading frame (ORF)

with homology to the *N. gonorrhoeae* iga gene sequence previously published (Pohlner, J. et al, Nature 325, 458-462 (1987)). The suggested iga gene from *H. influenzae* strain HK368 starting at the first ATG in the ORF as shown in Fig. 3 consists of 4646 nucleotides, including the TAA stop codon, encoding a deduced protein of 1541 amino acids. This primary translation product has a deduced molecular mass of 169 kd in contrast to the estimated size of the mature IgA1 protease of approximately 100 kd. In the discussion below is suggested a scheme for post-translational modifications of the preprotein to account for this discrepancy.

In connection with the present invention the iga gene encoding the IgA1 protease from the *H. influenzae* serotype b strain HK368 has been cloned and sequenced in order to characterize the protein further and thereby to achieve a better understanding of its role during the bacterial infection. The iga nucleotide sequence presented in Fig. 3 and the deduced primary structure of the IgA1 protease protein reveal several interesting features.

Potential start and stop signals for transcription and translation. The observed expression in *E. coli* implies that the *H. influenzae* iga gene must have transcription and translation signals which are recognized by the *E. coli* cell. A potential ribosome binding Shine-Dalgarno sequence 5'-TAAAGA -3' (nt 248 to nt 253 in Fig. 3) can be found eight nucleotides upstream from the putative translation start at the first ATG in the open reading frame (A at nt 262 in Fig. 3). This sequence has a homology of 5 identical positions out of 6 to the complementary sequence of the 3' end of the *E. coli* 16 S ribosomal RNA (Shine, J. and Dalgarno, 1, Proc. Natl. Sci. USA 71, 1342-1346 (1974)). In addition, it is located in an acceptable position according to the rules for gene

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beginnings suggested by Stormo et al., Nucl. Acids Res. 10, 2971-2996 (1982)). The sequence 5'-TAAACT-3' (nt 235 to nt 240) and 5'-TTGTG-3' (nt 211 to nt 215) might constitute the transcription signals at -10 and -35, respectively. The proposed -10 sequence is identical to the E. coli consensus sequence at the four best conserved positions (Hawley, D.K. and Mc Clure, W.R., Nucl. Acids Res. 11, 2237-2255 (1983)).

10 Located 19 base pairs upstream from this, the -35 sequence is within the allowed spacing and has a homology of 3 out of 5 positions identical to the E. coli consensus sequence (Hawley, D.K. et al., supra). The identification of these three putative promotor elements strongly support the suggestion of translational initiation at nt 262.

The ORF is ended by two sequential TAA stop codons at nt 4885 to nt 4890. A sequence similar to a typical Rho-independent transcription terminator is found at nt 4919 to nt 4943 and contains a perfect inverted repeat with the potential of forming a "hairpin structure", having a loop of 5 nt and a stem of 10 nt including five pairs of CG (Fig. 3). Like the typical E. coli terminators, this sequence structure is followed by a stretch rich in T residues (Rosenberg, M. and Court, D., Ann. Rev. Genet. 13, 314-353 (1979)).

Codon usage. Table 1 shows the codon usage of the H. influenzae iga gene. There is a striking tendency for the triplets to end preferentially with A or T. This may be a simple reflection of the high A+T content of the genome (mole percent A+T = 62). The codon frequencies in the iga gene are similar to those observed in four other H. influenzae genes for which the nucleotide sequence is known (Chandrasegaran, s. et al., Gene 70, 387-392 (1988)). A mole percent of 63% A+T was found for the 4626

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nt *H. influenzae* iga gene sequenced, which is close to the published estimate of 62% A+T for the whole genome (Kilian, M., J. Gen. Microbiol. 93, 9-62 (1976)). According to the observations of Bibb et al., Gene 30, 157-166 (1984), genes of this genome should have an A+T content of 51%, 65%, and 69% for the first, second, and third position within codons. Values of 53%, 61%, and 75% A+T were found at these positions.

Based on the preference of T over C in the third base of duet codons and the relatively low quartet to duet frequency ratios among sextet codons encoding Arg, Leu, and Ser, the iga gene should be weakly expressed according to the rules of Grantham et al., Nucl. Acids Res. 9, 43-74 (1981). This agrees well with the relatively low amount of IgA1 protease protein produced by *H. influenzae*.

This bias towards A and T residues of the third position of degenerate codons in the *H. influenzae* iga gene is in contrast to the markedly unbiased codon usage for the iga gene from *N. gonorrhoeae* which has a genomic A+T content of 51%.

These two genes most likely have a common ancestor indicating that apparently different constraints in codon usage have worked during the evolution of these two organisms.

Comparison of *H. influenzae* and *N. gonorrhoeae* protease sequences. Koomey and Falkow, Infect. Immun. 43, 101-1-7 (1984) have previously shown that the cloned type 2 *N. gonorrhoeae* iga gene hybridizes to the *H. influenzae* type 1 iga gene. The present comparison of the nucleotide sequences of these two iga genes revealed regions with a high degree of homology as well as areas with essentially no homology. The maximal homology between the

corresponding deduced amino acid sequences of the two IgA1 protease proteins was obtained by aligning them as shown in Fig. 4.

5 Polner et al., Nature 325, 458-462 (1987) found that the precursor of the *N.gonorrhoeae* IgA1 protease contains three functional domains; the amino-terminal signal peptide, the central IgA1 protease, and the carboxy-terminal "helper" domain. The leader peptide is released
10 during translocation of the preprotein into the periplasmic space while the helper domain is assumed to create a pore in the outer membrane for extretion of the protease domain and remains associated with the membrane upon autoproteolytic cleavage.

15 The primary translation product, deduced from the *H. influenzae* iga sequence presented in Fig. 4, has a molecular mass of 169 kd. The mature type 1 IgA1 protease from *H. influenzae* has been estimated to be about 100 kd.
20 Grundy et al. (*J. Bacteriol.* 169, 4442-4450 (1987)) found that a 2.2 to 3.1 kb region of the 3' end of a type 1 IgA1 protease gene from a *H. influenzae* serotype d strain is necessary for the secretion of the protease but not for its activity. They suggest that this region is cleaved off
25 during maturation of the protease. Based on these observations and the amino acid sequence homologies shown in Fig. 4, it is suggested that the *H. influenzae* IgA1 protease is secreted by a mechanism similar to the one proposed by Polner et al., supra, for the *N. gonorrhoeae*
30 IgA1 protease.

The aminoterminal part of the *H. influenzae* IgA1 protease contains positively charged lysines at amino acid (aa) positions 4, 5 and 7 followed by a hydrophobic stretch
35 including the helix breaking proline at position 21 four residues before an alanine (Fig. 4). These features are

- 17 -

characteristic of signal sequences which are cleaved off and released with the alanine as the C-terminal amino acid, (Watson, M.E.E., Nucl. Acids Res. 12, 5145-5164 (1984)). The two protease sequences aligned as shown in Fig. 4 are identical around the position of the cleavage site of the *N. gonorrhoeae* protease leader peptide determined by Polner et al., supra. Therefore it is proposed that the 25 amino terminal amino acids of the *H. influenzae* IgA1 protease preprotein constitute the signal peptide.

Sequences rich in prolines, much like the type 1 IgA1 protease target site in the hinge region of the human IgA1 molecule (Fig. 1), are exclusively found at three positions in the *H. influenzae* protease sequence (A, B, and D in Fig. 4). A single sequence with homology to the type 2 IgA1 protease target site (Fig. 1) is found in the same region of the protease (C in Fig. 4). It is proposed that one or more of these four sequence elements are functionally equivalent to the autoproteolytic sites a, b, and c in the *N. gonorrhoeae* protease, as shown in Fig. 4.

When an N-terminal signal sequence of 25 amino acids is taken into account, autoproteolytic cleavage at positions A, B, C or D would result in excreted proteases of deduced molecular masses of 107.8, 108.1, 109.9, or 110.3 kd, respectively. Each of these values are in acceptable agreement with the estimated Mr of 100 kd.

Strikingly, a stretch of 32 amino acids (aa 16 to aa 47) is identical in the two proteases except for a single conservative substitution. This shows that the N-terminal part of the mature IgA1 protease has been evolutionary conserved suggesting that it is essential to the enzymatic function or specificity of the protease molecule.

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Another notably well-conserved sequence is found at aa 785 to aa 797. It contains the two conserved cysteines which Polner et al.,supra, proposed to be part of the active site in the N.gonorrhoeae protease. A third cysteine is found in the "helper" domain of the H. influenzae protease preprotein at aa position 1250.

In striking contrast to the rest of the H. influenzae IgA1 protease, the region from aa 980 to aa 1240 has no significant sequence homology to the N. gonorrhoeae protease (see Fig. 4). This stretch is proposed to constitute the N-terminal part of the helper domain. It is very hydrophilic in both proteins suggesting that it has a common function in the secretion of the two proteases. No evolutionary deletions or insertions had to be introduced in this divergent area of the two protease sequences to obtain the flanking homologies as shown in Fig. 4. This observation is in contrast to the results of Grundy et al. who found a deletion-substitution loop in this region when analyzing DNA heteroduplexes formed between cloned H. influenzae type 1 and H. influenzae type 2 IgA1 protease genes.

The cloning and sequencing of the IgA1 protease gene from H. influenzae serotype b provides a tool to produce this protein in large quantities for further structural and functional analyses.

As mentioned, the IgA1 protease in question is primarily useful as an active component in vaccines for immunizing against bacterial meningitis. A vaccination against infections of the type concerned is in principle best performed by administering the vaccine via the oral cavity so that the vaccine gets into contact with the lymphoid tissues in the intestinal wall. Examples of administration of the vaccines are a) presentation on the surface of

transformed E.coli or other suitable host (vide infra), b) incorporated in micropheres alone or together with other suitable vaccine components or immunostimulant, and c) as part of a fusion protein with the subunit B of the cholera toxin. Administration of the vaccines may also be by the parenteral route. These examples are provided to describe and not to limit the possible applications.

The fact that the signals for expression of IgA1 gene and that the secretion mechanisms of the H. influenzae IgA1 proteases function in E. coli imply that the IgA1 protease gene has potential use as a vector for producing foreign proteins in cultures of E. coli or other suitable hosts.

The following examples are provided to describe this aspect in further detail. The examples are intended to illustrate and not to limit the invention:

Changes in the structural part of the iga gene do not affect secretion of the protein from the surface of the transformed E.coli clone. Insertion of prokaryotic or eukaryotic gene sequences encoding foreign polypeptides into IgA1 protease gene results in the transport to the E.coli cell surface of a hybrid protein. The hybrid protein may be exposed and retained on the cell surface or released by autoproteolysis into the growth medium depending on the location and size of the foreign gene insert. Such transformed E.coli clones or other suitable hosts exposing or releasing such fusion proteins on or from the cell surface may be used as an oral vaccine for presentation of one or more vaccine polypeptides to relevant lymphoid tissues in the gut of humans or animals. Alternatively, such clones may be employed for production of foreign polypeptides in E.coli or other hosts. Release into the medium may, when necessary, be accomplished by addition of active IgA1 protease.

Detailed Description of Aspect 2:Bacterial strains

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A collection of 133 isolates of *Neisseria meningitidis* recovered from 97 patients and 36 healthy carriers in 19 countries was examined. The 133 isolates represented 88 multilocus enzyme genotypes (ETs) defined on the basis of electrophoretically detectable allelic variations in 15 genes encoding enzymes (Caugant, d. et al., J. Bacteriol. 169, 2781-2792, 1987; Infect. Immun. 56, 2060-2068, 1988). In addition, 8 gonococcal strains, 4 each of the IgA1 protease types 1 and 2, were examined. IgA1 protease preparations produced by the method described by Higerd, T.B. et al. (J. Immunol. Methods 18, 245-249, 1977) from four meningococcal strains and one gonococcal strain were used to immunize rabbits by a method described previously (Kilian, M. et al. Molec. Immunol. 20, 1051-1058, 1983). The resulting antisera were examined for their ability to inhibit IgA1 proteases from all 133 meningococcal and 8 gonococcal strains using methods described (Kilian, M. et al., supra). While three of the four antisera against meningococcal proteases and the antiserum against gonococcal IgA1 protease inhibited IgA1 proteases from only some of the 141 strains, the remaining antiserum surprisingly caused inhibition of all IgA1 proteases whether of type 1 or 2 and whether of meningococcal or gonococcal origin. The inhibition titer determined against representative IgA1 protease preparations adjusted to standard activity as described (Kilian, M. et al. supra) ranged from 1024 to 4096. The meningococcal strain (HF13) used for production of this antiserum was of serogroup Y, subtype 2c, and produced an IgA1 protease of type 2. Identical IgA1 proteases were identified in other meningoccal strains of subtype 2c.

The IgA1 protease gene from *Neisseria meningitidis* strain HF13 was cloned in *E.coli* using methods described above for the cloning of the *H.influenzae* iga gene. A map of the gene and adjoining genome areas in phage lambda L47.1 is shown in fig. 5. The transformed *E.coli* clone secreted the meningococcal IgA1 protease into the growth medium and the resulting IgA1 protease isolated from the medium was capable of inducing neutralizing antibodies in rabbits with identical inhibition spectrum as antibodies raised against the IgA1 protease of the parental meningococcal strain HF13. These results show that the IgA1 protease isolated from meningococcal strain HF13, from a strain producing an identical or similar IgA1 protease, or from *E.coli* or other suitable host transformed with the iga gene, is a candidate for a vaccine against all types of meningococci and gonococci.

Detailed Description of Aspect 3:

The mentioned extensive IgA degradation in nasopharyngeal secretions of children with allergic diseases suggests that IgA1 protease-induced cleavage of IgA is a contributing factor to the development and perpetuation of allergic diseases. Two possible explanations might explain the more extensive IgA cleavage seen in allergic subjects: 1) an increased colonization by IgA1 protease-producing bacteria in the nasopharynx and 2) an inability of allergy-prone subjects to react against bacterial IgA1 proteases with enzyme-neutralizing antibodies.

To study this, the nasopharyngeal flora of 24 children was examined at the age of 1 year and the age of approximately 30 months. Representative bacterial isolates were identified and examined for IgA1 protease activity using standard methods. All children were examined clinically

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and immunologically to establish a possible diagnosis of allergic diseases.

5 Out of the 24 children, a total of 11 had developed allergic diseases at the age of 1 year. Table 1 correlates the clinically and immunologically established allergy diagnosis with the bacteriological findings. The figures demonstrate a significantly higher proportion of IgA1 protease-producing bacteria in nasopharyngeal samples from
10 allergic children. Surprisingly, the numerically most significant IgA1 protease-producer was Streptococcus mitis biovar 1. This organism has previously been a member of "Streptococcus mitior" (Kilian, M., Mikkelsen, L., & Henrichsen, J. Int. J. Syst. Bacteriol. 39, 471-484,
15 1989). This organism occurs in an IgA1 protease-producing form and in a form not producing this enzyme. In accordance with the findings in the control group, previous studies have demonstrated that S.mitis biovar 1 normally occurs in the form lacking IgA1 protease activity in the
20 nasopharynx (Kilian, M. & Holmgren, K. Infect. Immun. 31, 868-873, 1981). At the examination 18 months later, samples from allergic children contained Streptococcus pneumoniae and Streptococcus oralis in addition to
25 bacteria listed in Table 1. These data demonstrate that children developing allergic diseases are colonized with IgA1 protease-producing bacteria in the nasopharynx to a higher extent than healthy children. A vaccine containing IgA1 proteases from the relevant bacteria mentioned is likely to secure that the immune barrier is left intact,
30 thus preventing the development and perpetuation of allergic diseases.

TABLE 1

IgA1 protease producing bacteria as percentage of total bacterial flora in pharynx of 24 1 year old children with or without allergic diseases

IgA1 protease-producing bacteria (% of total flora)

Subject	All species	H.influenzae	H.parahaemolyticus	S.mitis biovar 1	N.meningitidis
A	30%	0	0	30	0
I	38%	2.4	0	33.4	2.2
N	64%	1.0	0	63	0
A	36%	0	2	34	0
+Allergy	52%	5.1	0	46.9	0
a	58%	0.4	0	0	57.6
D	14%	0	0	14	0
O	38%	0	0	38	0
R	18%	0	0	18	0
Æ	31%	23	0	8	0
Ø	22%	11.7	0	8.3	0
Median	36%	0.4%	0	30%	0
(range):	(14-64%)	(0-23%)	(0-2%)	(0-63%)	(0-57.6%)

TABLE 1 (continued)

IgA1 protease producing bacteria as percentage of total bacterial flora in pharynx of 24 1 year old children with or without allergic diseases

IgA1 protease-producing bacteria (% of total flora)

Subject	All species	H.influenzae	H.parahaemolyticus	S.mitis biovar 1	N.meningitidis
K	8%	0.05	0	8	0
M	6%	0	0	6.4	0
P	4%	0	0	4	0
T	2%	1.2	0	0.8	0
Z	2%	0.1	0	1.9	0
b	14%	0	3.6	10.4	0
-Allergy C	2%	0	0	0	2
E	0.4%	0.4	0	0	0
F	14%	2.1	0	11.9	0
H	0.8%	0.8	0	0	0
L	2%	0	0	2	0
U	10%	0.4	0	9.6	0
V	10%	1.1	2.2	6.7	0
Median	4%	0.1%	0	4%	0
(range):	(0.4-14%)	(0-2.1%)	(0-3.6%)	(0-10.4%)	(0-2%)

Explanation to the figures.

Fig. 1. Primary structure of the hinge region of human IgA1. The arrows indicate the peptide bonds cleaved by the individual IgA1 protease.

Fig. 2. Structure of the H. influenzae serotype b IgA1 protease gene. A Restriction maps of the analysed recombinant lambda phages containing sequences with homology to the H. influenzae serotype d iga gene probe. Symbols: (E) EcoRI; (H) HindIII; terminal bar indicates left arm of the individual phages; (+) or (-) indicate presence or absence of human IgA1 cleaving activity in culture supernatants of E. coli harbouring the individual clones. B Restriction fragments from EcoRI/HindIII double digests hybridizing to the two serotype d iga gene probes. The deduced orientation of the iga gene is indicated by 5' and 3'. C Combined restriction map of λ 368iga-8 and λ 369iga-16, an extension of A by including the restriction enzymes BamHI (B), ClaI (C), and PstI (P). D Restriction fragments of λ 368iga-16 subcloned into M13mp19 for sequence analysis.

Fig. 3. Nucleotide sequence of the cloned iga gene from H. influenzae serotype b strain HK368. The sequence shown is derived from a part of the 7.5 kb EcoRI-HindIII fragment of the λ 368iga-16 insert. The deduced amino acid sequence of the large ORF is shown above the nucleotide sequence. The two sequential stop codons are indicated by asterisks. Putative -35 and -10 promoter elements as well as a possible Shine-Dalgarno (SD) sequence are overlined. The possible Rho-independent dual terminator is indicated by divergent arrows.

Fig. 4. Amino acid homologies between the deduced H. influenzae iga gene product (H11GAP) and the N. gonorrhoeae

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iga gene product (NG1GAP). Sequence data of *N. gonorrhoeae* iga was derived from Pohlner et al., supra. Asterisks denote identical or functionally equivalent residues. Gaps indicated by dashes are introduced in the two sequences to obtain maximal homology. Cleavage site for the amino terminal signal peptide of the *N. gonorrhoeae* protease is indicated by s; S denotes the proposed equivalent cleavage site of the *H. influenzae* protease precursor. Positions a, b, and c represent autoproteolytic sites for the *N. gonorrhoeae* protease; positions A, B, C, and D indicate similar autoproteolytic sites suggested for the *H. influenzae* protease.

Fig. 5. Physical map of lambda HF13iga-1 expressing the meningococcal IgA1 protease gene from strain HF13. Symbols: E, Eco RI; H, Hind III; Xb, Xba I; Xh, Xho I. Hatched bar, fragment hybridizing to the 4.3 kb Hind III fragment of pVD 105 which contains the major part including the 3' end of the *N. gonorrhoeae* iga gene (Koomey, J.M., and Falkow, S., 1984 supra). The enzymes BamHI and SalI were found not to cleave the lambda HF13 iga-1 DNA.

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C l a i m s:

1. Immunoglobulin A1 protease (IgA1 protease) and
5 fragments thereof, characterized in that it
is produced by cloning the gene for the IgA1 protease from
a microorganism in a host organism, preferably selected
among *Escherichia coli*, *Salmonella* and *Saccharomyces*, from
10 which the IgA1 protease formed is secreted extra-
cellularly.
2. IgA1 protease according to claim 1, characterized
15 in that it is produced by cloning in an *E. coli* host organism.
3. IgA1 protease according to claim 1 or 2,
characterized in that the cloned gene
originates from *Haemophilus influenzae* serotype b.
- 20 4. Method of producing IgA1 protease according to claim
3, characterized in that an expression vector
containing a gene coding for the IgA1 protease in question
is introduced into an *E. coli* host organism, the gene
25 originating from *Haemophilus influenzae* serotype b HK368,
that the host organism is cultured to produce the IgA1
protease and that the protease is isolated from the
culturing medium in a manner known per se.
- 30 5. Gene for use in an expression vector in the method
according to claim 4, characterized in that
it has the nucleotide sequence shown in Fig. 3, wherein SD
represents a possible Shine-Dalgarno sequence.
- 35 6. Vaccine for immunizing against bacterial meningitis,
characterized in that it contains IgA1
protease from *Haemophilus influenzae* serotype b or

fragments thereof according to claim 3, and optionally IgA1 protease from *Neisseria meningitidis* and/or *Streptococcus pneumoniae*.

5 7. Vaccine according to claim 6, characterized in that it is formulated for peroral use.

8. Vaccine for the prevention of gonorrhoea and meningococcal meningitis, characterized in that it
10 contains IgA1 protease from *Neisseria meningitidis*.

9. Vaccine for the prevention or treatment of allergic diseases, characterized in that it contains IgA1 proteases from the bacteria which as a result of
15 their cleavage of the mucosa antibodies are responsible for the allergic reaction.

10. Vaccine according to claim 9, characterized in that the IgA1 proteases originate from bacteria
20 selected among *Haemophilus influenzae*, *H. parahaemolyticus*, *Streptococcus pneumoniae*, *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus mitis* biovar 1 and *Neisseria meningitidis*.

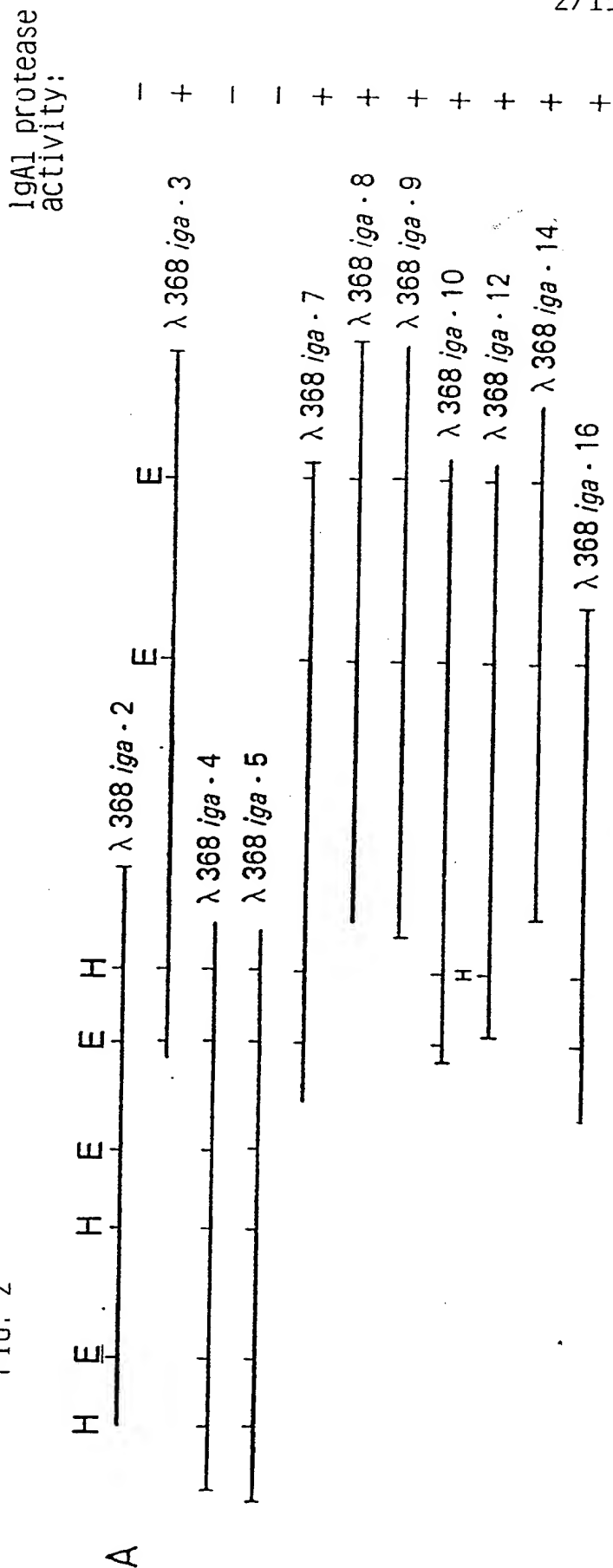
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FIG. 2



SUBSTITUTE SHEET

5' 3'

BE H C P EP C E P

H E

+ - -



CATCATTACGCCTTAATTAAACCAAAAGTGCGGTAAAAAATAGCCCAATTTTTTAAAAAT
10 20 30 40 50 60

ATCAAAAATCATTATCATTACCTCGTAATGTAATTCAAGTTTTGAGCGATTTATGCTAT
70 80 90 100 110 120

AAACCCCACTCATTATAAAAATGAAGACAACCTTGCAACTATCAATGCAACACAGCCAAG
130 140 150 160 170 180

ATTGGAATCAACTTGTAAGCCGTATATCAAAATTGTGTCTATCAATCTACTTTTTAACT
190 200 210 220 230 240

-35 -10

SD

TAATTAATAAGACAGCTTCTATGCTAATAAAAATTCAAACTCAATTTTATTGCACTT
250 260 270 280 290 300

T U A Y A L T P Y T E A A L U A D D U D
ACTGTCGCCTACGCATTAAACCCCTTATACAGAAGCCGCTTAGTGAGAGACGATGTGGAT
310 320 330 340 350 360

Y Q I F A D F A E H K G K F S U G A T H
TATCAATATTCGGTGATTTTGCAGAGAATAAAGGGAGTTTTCTGTTGGTGCAACAAAT
370 380 390 400 410 420

U L U K D K H H K D L G T A L P H G I P
GTGCTGGTAAGGATAAAAATAAAGATTGGGCACTGCCTTACCTAACGGTATTCCG
430 440 450 460 470 480

M I D F S U U D U D K A I A T L I H P Q
ATGATTGATTTTAGCGTGGTAGATGTAGATAACGCATTGCCACATTGATAATCCACAA
490 500 510 520 530 540

Y U U G U K H U S H G U S E L H F G H L
TATGTAGTAGGTGTAAACACGTTAGTAACGGCGTGAGTGAACACTACATTTTGGTAACTTA
550 560 570 580 590 600

H G H H H H G H A K A H A D U S S E E H
AACGGCAATATGAATAATGGCAATGCCAAGGCACACCGAGATGTATCTTCAGAGAAAT
610 620 630 640 650 660

FIG. 3 (continued)

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R Y F S U E K H E Y P T K L H G K T U T
AGATATTTTCCGTTGAGAAAAATGAGTATCCAACCTAATTGAATGGAAAAACAGTAACT
670 680 690 700 710 720

T E D Q T Q K R R E D Y Y H P R L D K F
ACGGAGATCAAACTCAAAAACGCCGTGAAGACTACTATATGCCACGTCTTGATAAATTT
730 740 750 760 770 780

U T E U A P I E A S T A S S D A G T Y H
GTTACGGAGGTTGCACCAATAGAGGCTTCAACCGCAAGTAGTGATGCTGGCACATATAAT
790 800 810 820 830 840

D Q N K Y P A F U A L G S G S Q F I Y K
GATCAGAATAAATATCCTGCTTTTGTAGACTAGGAAGTGGTAGTCAATTTATTTATAAA
850 860 870 880 890 900

K G D N Y S L I L N N H E U G G H N L K
AAAGGAGATAATTACAGCTTAATTTTAATAATCATGAGGTTGGAGGCAATAATCTTAA
910 920 930 940 950 960

L U G D A Y T Y G I A G T P Y K U N H E
TTGGTGGGCGATGCCTATACCTATGGTATTGCAGGTACACCTTATAAGTAARCCACGAG
970 980 990 1000 1010 1020

H N G L I G F G H S K E E H S D P K G I
AATAATGGACTAATTGGTTTTGGCAATTCAAAAGAGGAACACAGCGATCCAAAAGGARTA
1030 1040 1050 1060 1070 1080

L S Q D P L T N Y A U L G D S G S P L F
TTATCTCAGATCCTCTTACCAATTATGCTGTTTTAGGCGACAGTGGCTCCCCATTATTT
1090 1100 1110 1120 1130 1140

U Y D R E K G K W L F L G S Y D F W A G
GTATATGATAGAGAAAAAGGAAAATGGCTTTTTCTTGGGTCTTATGATTTTTGGGCGGGT
1150 1160 1170 1180 1190 1200

Y N K K S W Q E W N I Y K S Q F T K D U
TATACAAAAATCTTGGCAAGATGGATATTTATAAATCTCAATTTACTAAAGATGTT
1210 1220 1230 1240 1250 1260

L H K D S A G S L I G S K T D Y S W S S
CTCAATAAGATAGTGCAGGTTCTTTAATTGGTTCCAAGACAGATTATAGTTGGTCTTCT
1270 1280 1290 1300 1310 1320

H G K T S T I T G G E K S L N U O L R O
AATGGCAAGACAAGTACGATTACGGGAGGGGAGAAATCTTTAARTGTTGATTTAGCTGAC
1330 1340 1350 1360 1370 1380

G K O K P N H G K S U T F E G S G T L T
GGAAAAGATAAACCTAATCACGGGAAGAGTGTACATTTGAAAGGGAGTGGAAACGCTTACC
1390 1400 1410 1420 1430 1440

L N H N I D Q G A G G L F F E G D Y E U
TTAATAATAATATCGATCAAGGTGCAGGCGGATTATTCTTTGAAGGCGATTATGAAGTT
1450 1460 1470 1480 1490 1500

K G T S O H T T W K G A G U S U A E G K
AAGGTACTTCTGATAATACTACTTGGAAAGGAGCAGGTGTCTCTGTTGCCGARGGAAAA
1500 1520 1530 1540 1550 1560

T U T W K U H H P Q Y D A L A K I G K G
ACTGTAACTGGAAAGTGCATAATCCTCAATATGATCGTTTAGCAAAATTTGGCAAGGG
1570 1580 1590 1600 1610 1620

T L I U E G T G D H K G S L K U G O G T
ACATTAATTGTTGAAGGAACAGGAGATAATAAGGTTTCGCTAAAGTGGGCGATGGCACC
1630 1640 1650 1660 1670 1680

U I L K Q Q T H G S G Q H A F A S U G I
GTTATTTTAAACAACAACAATGGTTTCGGGACACACGCTTTTGCTTCTGTAGGGATT
1690 1700 1710 1720 1730 1740

U S G R S T L U L N D O K Q U O P H S I
GTAAGTGGTTCGCTCAACTCTTGCTTAATGATGATAACAAGTAGATCCAATTCATT
1750 1760 1770 1780 1790 1800

Y F G F R G G R L D L N G H S L T F D H
TACTTTGGCTTTAGAGGCGGTTCGATTAGACTTAACGGTAATTCCTAACCTTTGATCAC
1810 1820 1830 1840 1850 1860

I R N I O D G A R L U N H H N T H A S H
ATCAGAAATATTGATGATGGTGCAGACTAGTTAATCATAATATGACTAATGCCTCAAT
1870 1880 1890 1900 1910 1920

I T I T G E S L I T O P H T I T P Y H I
ATAACGATTACTGGGGAAGTCTAATTACAGATCCAATACATTACTCCATATAATATA
1930 1940 1950 1960 1970 1980

O A P O E O N P Y A F R R I K O G G Q L
GACGCACCGATGAAGATAATCCTTATGCCTTTCGACGGATTAAAGATGGAGGACAGCTC
1990 2000 2010 2020 2030 2040

FIG. 3 (continued)

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Y L N L E N Y T Y Y A L R K G A S T R S
TATTTAATTTGGAAATTACACTTATTATGCGTTAAGAAAAGGTGCGAGCACTCGTTCA
2050 2060 2070 2080 2090 2100

E L P K N S G E S N E N W L Y N G K T S
GAATTACCTAAAAATAGTGGCGAAGCAATGAAATTGGCTATATATGGGTAAACTTCC
2110 2120 2130 2140 2150 2160

D E A K R N U N N H I N N E R M N G F N
GATGAAGCCAAAGAAATGTAATGAACCATATCAACAACGAGCGTATGAATGGCTTTAAT
2170 2180 2190 2200 2210 2220

G Y F G E E E G K N H G N L N U T F K G
GGTTATTTTGGCGAGGAAGAGGGTAAAAATAACGGTAATCTAATGTGACTTTTAAAGGC
2230 2240 2250 2260 2270 2280

K S E Q N R F L L T G G T H L N G D L T
AAAGTGAGCAAATCGCTTTTTATTACAGGCGGTACAACCTTAATGGCGATTTAACG
2290 2300 2310 2320 2330 2340

U E K G T L F L S G R P T P H A R D I A
GTTGAAAAGGCACCTTATTCCTTTTCAGGCAGACCAACACCGCACGCAAGAGATATTGCA
2350 2360 2370 2380 2390 2400

G I S S T K K D P H F R E N N E U U U E
GGTATTTCTTCGACAAAAAAGATCCTCACTTTGCTGAAATAATGAAGTGGTAGTAGAA
2410 2420 2430 2440 2450 2460

D D W I N R N F K A T T N N U T G N A S
GATGACTGGATTACCGCAATTTTAAGCAACCAATGAACGTGACTGGCAATGCCTCA
2470 2480 2490 2500 2510 2520

L Y S G R N U A N I T S N I T A S H K A
CTTTATTCAGGTGCAATGTTGCAACATTACGTCAATATCACAGCTTCTAATAAGCA
2530 2540 2550 2560 2570 2580

Q U H I G Y K T G D T U C U A S D Y T G
CAAGTTCATATCGGCTATAAAACAGGCGATACCGTTTGTGTACGTTCTGACTATACGGGC
2590 2600 2610 2620 2630 2640

Y U T C T T D K L S D K A L N S F N P T
TATGTGACTTGTACTACTGACAAATTATCCGATAAAGCCCTTAATAGCTTTAATCCRACC
2650 2660 2670 2680 2690 2700

N L R G N U N L T E S A N F U L G K A N
AATCTACGCGGCAATGTAATTTAACCAGAAAGTGCAAACTTTGTCTTAGGCAAGCCAAC
2710 2720 2730 2740 2750 2760

L F G T I Q S R G H S Q U A L T E N S H
TTATTTCGGCACAATTCAAGCAGAGGAATAGCCAGTACGTTTAAACCGAAATAGCCAT
2770 2780 2790 2800 2810 2820

W H L T G N S D U H Q L D L A H G H I H
TGGCATTTAACAGGAATAGTGATGTTTCATCAATTAGATCTAGCAATGGGCATATTCAT
2830 2840 2850 2860 2870 2880

L H S A D N S N H U T K Y N T L T U H S
TAAATTCAGCAGACAATTCAACAATGTGACAAATATAACACGCTGACTGTGAATAGC
2890 2900 2910 2920 2930 2940

L S G H G S F Y Y L T D L S N K Q G D K
TTATCAGGTAACGGTTCTTTCTATTATTTAACTGATCTTTCCAATAACAAGGCGACAA
2950 2960 2970 2980 2990 3000

U U U T K S A T G H F T L Q U A D K T G
GTTGTTGTAACATAATCCGCCACAGGTAACCTTACATTACAAGTGGCAGATAAACAGGC
3010 3020 3030 3040 3050 3060

E P H H H E L T L F D A S K A Q A D H L
GAGCCAATCATAATGAACCTCACACTTTTGTATGCTTCAAAAGCTCAAGGAGATCATTG
3070 3080 3090 3100 3110 3120

H U S L U G H T U D L G R W K Y K L R N
ATGTGTCATTAGTTGGGAATACCGTTGATTTAGGTGCTTGGAATATAAATTACGTAT
3130 3140 3150 3160 3170 3180

U H G R Y D L Y H P E U E K R H Q T U D
GTTAATGGACGTTACGATTTGTATAACCCAGAGGTGGAAAAAGAAATCAAACTGTCGAT
3190 3200 3210 3220 3230 3240

T T H I T T P H H I Q A D U P S U P S H
ACGACAATATCACACACCTAATAATATTCAAGCTGATGTGCCTAGCGTACCAAGTAAC
3250 3260 3270 3280 3290 3300

H E E I A A U D E A P U P P P A P A T P
AATGAAGAAATAGCCCGTGTGATGAAGCACCAGTTCACCCACCTGCGCTGCTACCCA
3310 3320 3330 3340 3350 3360

S E T T E T U A E N S K Q E S K T U E K
TCAGAGACAACTGAACAGTGGCTGAAATAGTAAGCAAGAAAGTAAACAGTAGAGAA
3370 3380 3390 3400 3410 3420

H E Q D A T E T T A Q N R E U A K E A K
AACGAGCAAGACGCAACCGAGACAACAGCTCAAAATAGAGAAGTTGCAAAAGAAAGCTAA
3430 3440 3450 3460 3470 3480

FIG. 3 (continued)

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S H U K A H T Q T H E U A Q S G S E T K
TCRAATGTAAAAGCTAATACTCAAACAAATGAAGTAGCTCAAAGTGGAAGTGAAACCAAG
3490 3500 3510 3520 3530 3540

E T Q T T E T K E T A T U E K E E K A K
GAAACTCAACGACTGAAACAAAAGAACAGCTACGGTAGAAAAAGAGAAAGGCTAAA
3550 3560 3570 3580 3590 3600

U E T E K T Q E U P K U T S Q U S P K Q
GTAGAACAGAGAAACTCAAGAGTCCCTAAGTGACTTCTCAAGTGTCTCCGAACAG
3610 3620 3630 3640 3650 3660

E Q S E T U Q P Q A E P A R A E H D P T U
GAACAGTCTGAAACTGTTCAACCGCAAGCAGAGCCTGCTCGTGAAATGATCCGACTGTT
3670 3680 3690 3700 3710 3720

H I K E P Q S Q T H T T A D T E Q P A K
AATATAAAGAGCCTCAATCTCAACCAATACACAGCAGACACTGAACAACCTGCGAAA
3730 3740 3750 3760 3770 3780

E T S S H U E Q P U T E S T T U H T G H
GAGACTAGCTCAATGTTGAACAACCAAGTGACAGAAAGCACACAGTAACCACTGGAAAC
3790 3800 3810 3820 3830 3840

S U U E H P E H T T P A T T Q P T U H S
TCTGTAGTGGAATCCAGAGAATACACACCTGCTACAACTCAACCTACGGTTAATTCA
3850 3860 3870 3880 3890 3900

E S S H K P K H R H R A S U R S U P H H
GAAGCAGTAATAAGCCAAAGATAGACATAGAGAGAGTGTTCGCTCAGTTCCGCATAAT
3910 3920 3930 3940 3950 3960

U E P A T T S S H D R S T U A L C D L T
GTTGAACCAAGCTACACAGTAGCAACGATCGTTCTACAGTAGCATTGTGCGATCTCACA
3970 3980 3990 4000 4010 4020

S T H T H A U L S D A R A K A Q F U A L
AGTACAAACACAATGCGGTACTTTCTGATGCAAGGGCAAAAGCACAAATTTGTTGCATTA
4030 4040 4050 4060 4070 4080

H U G K A U S Q H I S Q L E H H H E G Q
AATGTGGGGAAGCAGTTTCTCAACATATTAGCCAGTTAGAATGAATAACGAGGGGCA
4090 4100 4110 4120 4130 4140

Y H U W U S N T S H N K H Y S S S Q Y A
TATARC GTTGGGTATCTAATACTTCAATGAACAAAATTATTCCTCAAGTCAATATCGT
4150 4160 4170 4180 4190 4200

R F S S K S T Q T Q L G W D Q T I S H H
CGTTT TAGTTCTAAAGTACGCAACTCAACTGGGTTGGGATCAACAATCTCAACAAT
1210 1220 1230 1240 1250 1260

U Q L G G U F T Y U R H S H H F D K A T
GTTCA GTTAGGTGGCGTGT TACTTATGTTTCGCAATAGTAACAAC TTTGATAAGGCAACA
1270 1280 1290 1300 1310 1320

S K H T L A Q U H F Y S K Y Y A D N H W
AGTAA AATACTCTAGCACAGTTAATTTCTATTCTAATATTATGCGGATAATCATTGG
1330 1340 1350 1360 1370 1380

Y L G I D L G Y G K F Q S K L Q T H H H
TATTTGGGCATTGATTTAGGCTACGGCAAGTTCCAAGCAATTACAAC TAAATCATAAT
1390 1400 1410 1420 1430 1440

A K F A R A H T A Q F G L T A G K A F H L
GCGAATTTGCTCGCCATACTGCACAA TTTGGTTTAACCGCAGGCAAGCATT TTAATCTT
1450 1460 1470 1480 1490 1500

G H F G I T P I U G U R Y S Y L S H A D
GGCAATTTTGGTATTACGCCAATAGTAGGCGTGC GTTATAGCTATTTATCAACGCTGAT
1510 1520 1530 1540 1550 1560

F A L D Q A R I K U H P I S U K T A F A
TTTGCATTAGATCAAGCTCGCATTAAAGTAATCCAATATCTGTCAAACAGCCTTTGCT
1570 1580 1590 1600 1610 1620

Q U D L S Y T Y H L G E F S U T P I L S
CAAGTTGATTTAAGTTATACTTATCACTTAGGCGAGTTTTCGTTACGCCAATTTTGTCT
1630 1640 1650 1660 1670 1680

A R Y D A H Q G S G K I H U H G Y D F A
GCTCGATATGATGCAACCAAGGCAGCGGAAAAATTAATGTAAATGGATATGATTTTGCT
1690 1700 1710 1720 1730 1740

Y H U E H Q Q Q Y H A G L K L K Y H H U
TACAACGTGGAAAACCAACAGCAATATAACGCAGGGCTTAATTTGAATATCATAATGTG
1750 1760 1770 1780 1790 1800

K L S L I G G L T K A K Q A E K Q K T A
AAATTAAGTCTAATAGGCGGATTAAACAAAGCGAARACAGCGGAAAACAAAAACTGCA
1810 1820 1830 1840 1850 1860

FIG. 3 (continued)

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E L K L S F S F *
GAATTAARACTAAGTTTTAGTTTTTAATAAGCCTGTTTGAATTAACGTTATAARCAACA
1870 1880 1890 1900 1910 1920

AGCCCTGTGTATTACAGGGCTTATTTTTGAATGAATTCAGTGATTAAAGTCCGGTGAAA
1930 1940 1950 1960 1970 1980

AATCAGCGCATTTTTTATTTTTAACGTAAAAACGCTGGAAATTTTTTCTCATATGCTGAG
1990 5000 5010 5020 5030 5040

ATTTTGTCTTCGTGCTGAAGGGTTAAACCGATATTATCTAAACCGTTTAGC
5050 5060 5070 5080 5090

FIG. 4

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S

50

MLNKKFKLHFIALTU--RYALTPYTEARLURDOUDYQIFROFAENKGKFSUGATH
* * * * *
MKAKAFKINHAISLSIFLAYALTPYSEARLURDOUDYQIFROFAENKGKFFUGATO

S

100

ULUKOKHKKDLGTALPHGIPMIDFSUUDUDKRIATLINPQYUUGUKHUSHGUSEL
* * * * *
LSUKHKGQHI GHALSH-UPMIDFSURDUNKRIATUUDPQYUSUKHAKAEUHTF

150

HFGNLHGMMHGHAKAHROUSSEENRYFSUEKHEY-PTKLNKGTUTTEOQTQKRA
* * * * *
YYGQYNG-----HNDUAKENEYRUUEQHNYEPHKAUGASHLG-----RL

200

EDYYMPRLDKFUTEUAPIEASTASSDAGTYHQNKYPAFUALGSGSQFIYKKGDHY
* * * * *
EDYHMAFNFKUTEUAPIAPTDAGGLOTYKDKHAFSSFURIGAGQQLUYEKGUYH

250

SLILHHEUGGHNKLUGDAYTYGIAGTPYKUHNNH-----GLIGFGHSKEEHS
* * * * *
Q----EGNEKGYDLROLSQAYRYAIGTPYKDIHIDQTMNTEGLIGFGHNKQYS

300

--DPKGILSQOPLTHYVULGDSGSPLFUYDREKKGKULFLGSYDFWAGYNKKSUQE
* * * * *
REELKQALSQDALTHYGULGDSGSPLFAFOKQKHQUVFLGTYYWAGYGKKSUQE

350

WHIYKSQFTKOUHKKDSAGSLIGSKTOYSUSSNGKTSTITGGEKSLHVDLAOGKO
* * * * *
WHIYKKEFADKIKQHDHAGTUKG-NGEHHUKTTGTHSHI----GSTAVALANNEG

400

KPHHGKSUTFEGSGTLTLHNNIDQGAGGLFFEGDYEUKGTSOHTTUKGAGUSUAE
* * * * *
DANHGOHUTFEDHGTULHQNINQGAGGLFFKGDYTUKGANHOITULGAGIDUAD

FIG. 4 (continued)

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150

GKTUTUKUHPQYDALAKIGKGTLEIUEGTGONKGSCLKUGDGTUCLKQQTNGSGQH
* * * * *
GKKUUUQUKHPHGDALAKIGKGTLEIHTGTUNQGGQLKUGDGTUCLKQKADOKKU

500

-AFASUGIUSGASTLULHODKQUOPHSIYFGFAGGALDLHGNSLTFOHIRNIDOG
* * * * *
QAFSQUGIUSGAGTLULHSSHQINPDHLYFGFAGGALDANGNCLTFEHIRHUDEG

550

ARLUNHHMTNASHITITGESLITOPHTITPYHI-DAPDEDNPYAF--RAIKOGGQ
* * * * *
ARIUNHNTDHASTITLTGKSLITNPHSLSVHSIQHDOYDEDDSYYYRPARPIPGQ

600

LYLNLNYTYVALAKGASTASELPKHSGESNEHLYMGKTSDEAKAHUMHHIHNE
* * * * *
KOLYYKHYRYVALKSGGALHAPMPENGUAENNDWIFMGYTQEERAKNANHKKHNA

650

AMNGFHGYFGEEEGK-NHGNLHUTFKGKSEQHAFLLTGGTHLHGDLTUEKGTFL
* * * * *
RIGDFGGFFDEENGKGHNGALHLNFMGKSAQHAFLLTGGANLHGKISUTQGNVLL

700

SGRPTPHARDIAGISSTKKOPHFARENHEUUEDDWIHANFKATTMHUTGNASLYS
* * * * *
SGRPTPHARDFUNKSSARKDAHFSKNHEUUFEDDWIHATFKAREIAUNQSASFSS

750

GRNUAHITSHITASNAQUHIGYKTGOTUCURSODYTGYUTCCTOKLSOKALNSFN
* * * * *
GRNUSOITAHITATONAKUHLGYKNGDEUCURSODYTGYUTCNTGHLSDOKALNSFO

800

PTNLRGHNLTESANFULGKAHLFGTIQSAGNSQUALTENSUHLTGNSDUHQLO
* * * * *
ATRINGHUNLHQHARLULGKAALWGKIQQGHSRUSLHQHSKUHLTGOSQUHLS

850

1350
 HYSSSQYRAFSSKSTQTQLGWQDTISHNUQLGGUFTYUANSNHFOKATSKHTLAQ
 * * * * *
 DYASAQYRAFSSKATQTQIGIDASLSEHMQIGGULTYSOSQHTFOQAGGKHTFUQ

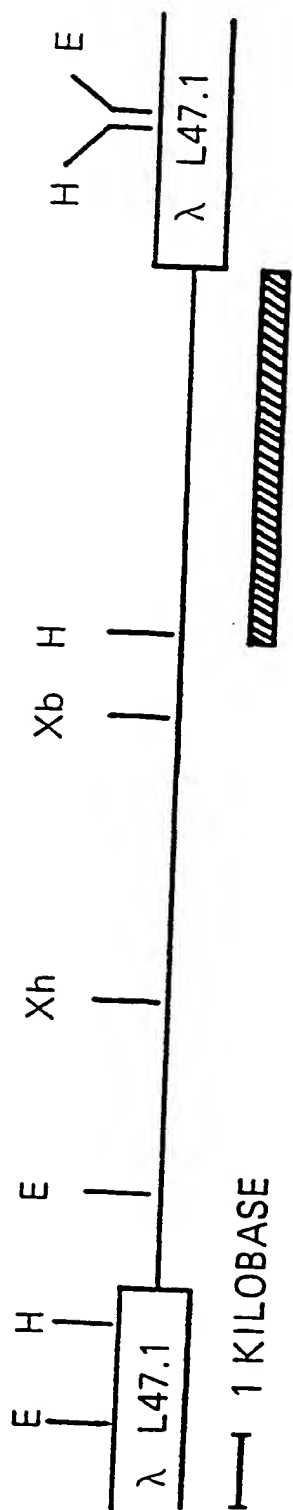
1400
 UHFYSKYAONHUYLGIDLGYGKFQSKLQTHHNAKFAAHTAQFGLTAGKAFHNGH
 * * * * *
 ANLYGKYYLNDWYUAGDIGAGSLASALQTQQKANFHATS IQTGLTLGHTLKIHQ

1450
 FGITPIUGURYSYLSHADFALOQARIKUNPISUKTAFQUOLSYTYHLGEFSUTP
 * * * * *
 FEIUPSAGIRYSRLSSADYKLGODSUKUSSMAUKTLTAGLOFAYRFKUGHLTUKP

1500
 ILSAAYDANQGSCKIHUNGYDFAYHVENQQQYNAGLKLKYHNUKLSLIGGLTKAK
 * * * * *
 LLSAAYFAHYGKGGUNUGGKSFAYKADNQQQYSAGUALLYAHUTLNUNGSITKCK

1540
 QAEKQKTAELKLSFSF HIIGAP
 * * * * *
 QLEKQKSGQIKIQIRF HGIGAP

FIG. 5



INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 90/00073

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 12 P 21/02, C 12 N 9/50, C 12 N 15/57, A 61 K 39/02 39/095											
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 10px;">Minimum Documentation Searched⁷</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 20%; padding: 5px;">Classification System</td> <td style="padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">IPC5</td> <td style="padding: 5px;">C 12 N; C 12 P</td> </tr> </table> <div style="text-align: center; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div>			Classification System	Classification Symbols	IPC5	C 12 N; C 12 P					
Classification System	Classification Symbols										
IPC5	C 12 N; C 12 P										
SE,DK,FI,NO classes as above											
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category</th> <th style="width: 60%; padding: 5px;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 30%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;"> Chemical Abstracts, volume 107, no. 21, 23 November 1987, (Columbus, Ohio, US), Grundy, Frank J. et al: "Haemophilus influenzae immunoglobulin A1 protease genes: cloning by plasmid integration-excision, comparative analyses, and localization of secretion determinants", see page 207, abstract 192238f, & J.Bacteriol. 1987, 169(10), 4442-44509 -- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;"> Chemical Abstracts, volume 104, no. 15, 14 April 1986, (Columbus, Ohio, US), Fishman, Y et al: "Cloning of the type 1 immunoglobulin A1 protease from Neisseria gonorrhoeae and secretion of the enzyme from Escherichia coli", see page 184, abstract 124204e, & Pathog. Neisseriae, Proc. Int. Symp., 4th 1984, 164(8), 9 -- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-6</td> </tr> </tbody> </table>			Category	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	Chemical Abstracts, volume 107, no. 21, 23 November 1987, (Columbus, Ohio, US), Grundy, Frank J. et al: "Haemophilus influenzae immunoglobulin A1 protease genes: cloning by plasmid integration-excision, comparative analyses, and localization of secretion determinants", see page 207, abstract 192238f, & J.Bacteriol. 1987, 169(10), 4442-44509 --	1-5	X	Chemical Abstracts, volume 104, no. 15, 14 April 1986, (Columbus, Ohio, US), Fishman, Y et al: "Cloning of the type 1 immunoglobulin A1 protease from Neisseria gonorrhoeae and secretion of the enzyme from Escherichia coli", see page 184, abstract 124204e, & Pathog. Neisseriae, Proc. Int. Symp., 4th 1984, 164(8), 9 --	1-6
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 50%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>											
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search 14th June 1990 </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report 1990 -05- 26 </td> </tr> <tr> <td style="width: 50%; padding: 5px;"> International Searching Authority SWEDISH PATENT OFFICE </td> <td style="width: 50%; padding: 5px;"> Signature of Authorized Officer Anneli Jönsson <i>stedt-jönsson</i> </td> </tr> </table>			Date of the Actual Completion of the International Search 14th June 1990	Date of Mailing of this International Search Report 1990 -05- 26	International Searching Authority SWEDISH PATENT OFFICE	Signature of Authorized Officer Anneli Jönsson <i>stedt-jönsson</i>					
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	<p>Chemical Abstracts, volume 99, no. 5, 1 August 1983, (Columbus, Ohio, US), Bricker, J. et al: "IgA1 proteases of Haemophilus influenzae: cloning and characterization in Escherichia coli K-12 ", see page 132, abstract 33850z, & Proc.Natl.Acad.Sci.U.S.A. 1983, 80(9), 2681-26859</p> <p style="text-align: center;">--</p>	1-6
X	<p>Chemical Abstracts, volume 98, no. 91, 28 February 1983, (Columbus, Ohio, US), Koomey, J. Michael et al: "Genetic and biochemical analysis of gonococcal IgA1 protease: cloning in Escherichia coli and construction of mutants of gonococci that fail to produce the activity ", see page 145, abstract 66414t, & Proc.Natl.Acad.Sci.U.S.A. 1982, 79(24),</p> <p style="text-align: center;">--</p> <p style="text-align: center;">-----</p>	1-6

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 90/00073

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 90-05-07
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date